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THE FLAGELLATE CHARACTER AND RECLASSIFICATION OF THE PARASITE PRODUCING "BLACKHEAD" IN TURKEYS—*HISTOMONAS* (GEN. NOV.) *MELEAGRIDIS* (SMITH)*

ERNEST EDWARD TYZZER

The demonstration of the constant presence in the parasite of Blackhead of an extranuclear body which takes part in nuclear division, and the occurrence of a type of nuclear division commonly found in trichomonads (Kofoid and Swezy, 1915) led to the suggestion in an earlier paper (Tyzzer, 1919) of its flagellate character. A subsequent series of observations, to be recorded further on in the present paper, show that this organism may exhibit under certain conditions characteristic flagellate motility.

In the paper above referred to, the author has called attention to the extreme pleomorphism of the protozoon in question, the extent of its morphological variation having no parallel among known parasitic amoebae. The distribution of the various forms of the parasite bears such a relation to the age of the pathological process with which they are associated that they may be interpreted as representing phases of development. Amoebiform organisms with clear blue staining cytoplasm, either with or without inclusions of the nature of ingested material, which occur in great numbers in early lesions and at the periphery of older lesions, were considered to be invasive forms. Organisms having a clear, faintly staining cytoplasm, frequently occurring in such numbers as to greatly distend the tissues and associated with the stage of the disease immediately following invasion were regarded as vegetative forms, although it is quite possible that a large proportion of such organisms present abnormal development and degeneration. A third type of organism found predominating in the older portions of the lesions was interpreted as representing a resistant phase of development. These are relatively small and rounded, present a dense cytoplasm having a marked affinity for eosin and possess a delicate limiting membrane. They are taken up in great number by giant cells.

The multiplication of this organism in the tissues appears to be accounted for by binary fission, at least no evidence of any other type of propagation has been obtained. The process of nuclear division is of the type found in trichomonads. The division centers are derived from an extranuclear body, and are connected with each other by a well developed rod-like paradesmose. No multinucleated forms have been found. Parasites with disk-like bodies in the cytoplasm have been interpreted by Smith (1915) as probably representing multiple agamic division. He evidently concludes that this species multiplies by a process which bears a striking similarity to the endogenous spore formation described in amoebae and Balantidium by Walker (1908). Organisms containing these disk-like inclusions are of common occurrence and have been carefully studied in the course of the present investigation. These inclusions vary in size from barely visible particles to bodies exceeding the nucleus in size, and are in some instances so uniform that the parent organisms bear considerable resemblance to the multinucleated cysts of entamoebae. Since the organisms in which they occur retain their original nucleus and extranuclear body unchanged, and since no evidence has been obtained that these disk-like bodies develop into mature organisms, it has appeared more probable that these inclusions represent either the shadows of ingested material or globules of coagulable material.

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The dissemination of the parasite from the primary lesions in the ceca to the liver of the turkey is accounted for by Smith through the transportation within phagocytic cells of the problematical small forms discussed above. It is supposed that these cells, after taking up the small forms of the parasite, may in certain instances pass into the veins when they are carried through the portal system to lodge in the liver. It is difficult on this hypothesis to account for the retention of the organisms in the liver, for it would be reasonable to expect that such small parasites on escaping from the phagocytic cells would be occasionally at least swept in the circulation to the lungs and other organs. That the liver serves as an effective filter for the parasite is shown by the limitation of the secondary lesions of the disease to this organ. The demonstration of an active invasive form of the parasite capable of migrating through any of the softer tissues makes the foregoing hypothesis unnecessary. The parasite infiltrates the involved tissue so extensively that it is inconceivable that the blood vessels should escape, and in fact, organisms have not infrequently been found beneath the endothelium of the veins. The size and physical peculiarities of these invasive forms evidently prevent their passage through the capillaries of the liver.

An attempt has been made by Hadley to show that the parasite of Blackhead is identical with "Trichomonas," an intestinal flagellate, which is treated as a species without further distinction. Apparently artefacts and postmortem changes are interpreted as evidences of invasion of the intestinal mucosa by intestinal flagellates. Not only are intestinal trichomonads incorporated into the life cycle of the parasite, but also organisms of the Blastocystis type. This author has not succeeded in demonstrating intermediate stages connecting any of the intestinal flagellates with the tissue parasite. The confusion of several intermingled species as the developmental forms of a single species has, strange to say, led him also to a conclusion which is not greatly at variance with what is now appparent concerning the nature of the parasite of Blackhead.

Amoeboid movement with slow change of shape has already been recorded (Tyzzer, 1919), but these earlier observations were made upon material kept in a warm chamber at 38 C., rather than at the body temperature of the turkey. Smith (1915) states that no motility has been observed in parasites studied from time to time in the warm chamber except on one occasion when slight changes of form were observed. During the present season, a series of observations made upon fresh material kept at temperatures ranging from 41 to 42 C., the body temperature of the turkey, has brought to light great activity in certain forms of the parasite. The organisms, in hanging drop preparations of scrapings from the lesions mixed with Locke's solution, remain motile for many hours at this temperature. Different types of organisms are distinguishable in fresh material. The small sized dense forms such as are encountered within giant cells are most conspicuous, but these are in general nonmotile. Large and moderate sized forms with a more or less granular cytoplasm frequently show motility, but others appear degenerated and consist of little more than a vesicle containing a granular mass in which a nucleus is at times distinguishable. Least conspicuous are the clear hyaline forms, and these show the greatest activity. The nucleus is usually distinguishable and an occasional organism is found in which four or five lines radiating from the extranuclear body are plainly visible. There is no sharp distinction between ectoplasm and endoplasm, but when granules are present, they are confined to the vicinity of the nucleus, leaving the cytoplasm otherwise clear.

The motility varies in type not only in different organisms, but in the same one at different times during the period of observation, and may be either amoeboid or pulsating in character. Furthermore, the amoeboid movements may be either continuous or interrupted. In the former case the motility may amount in some instances to a slow change of shape, in others to a more or less continuous flowing of the cytoplasm with snail-like progression. In case the amoeboid movement is discontinuous in type, pseudopodia are extended from the main mass of protoplasm, and the latter may stream into the former. There is extreme variation in the rate of motility. The pseudopodia may be sheet-like with a smooth border, but often show one or several rather sharp processes or spurs and are commonly quite irregular. Their protrusion is usually quite rapid, but not typically eruptive in character. The pseudopodia may be quickly protruded and retracted. An organism watched over a long period of time has been observed to spread out and become sheet-like. Several pseudopodia may be extended almost simultaneously from various points of the surface. Some large organisms may develop a number of short wave-like projections, but subsequently become rounded and remain quiescent. Some maintain a remarkable degree of activity for long periods of time, and amoeboid movement has been noted in organisms from a lesion kept at room temperature for forty-eight hours. Activity in a degree not observed in parasitic amoebae is frequently observed. Sudden displacement or rotation of the main body of organisms frequently results from the movements of extended pseudopodia or adjacent tissue cells may be forcibly separated and shoved to one side. Occasionally an organism exhibits for a considerable period, cytoplasmic movement of a wave-like character, in appearance suggestive of the boiling of a viscid material.

Rhythmic pulsating movements similar to that of trichomonads have been repeatedly observed. This was first noted in a lesion which had been left over night in a partially dissected turkey. Since the possibility of contamination with intestinal flagellates could not be excluded, this observation was disregarded. Organisms showing similar rhythmic movements were subsequently noted in secondary lesions removed with aseptic precautions from several different birds. Stained sections of these lesions reveal no organisms having the morphology of the common intestinal flagellates. Pulsation was in no case immediately apparent in material obtained from freshly killed birds, but developed after a period of from two to four hours in the warm chamber. Several organisms showing this type of motility were in one instance found with the low power from the movement imparted to surrounding cells and debris. They had the morphology of other typical Blackhead parasites present in the material, but were rotating in a jerky manner. No well developed undulatory membrane or flagella were apparent and the rhythmic movement appeared to be internal. On another occasion an organism was observed from which were protruded rather sharp wave-like pseudopodia. These after a time travelled in one direction over the surface of the body, the movements then became characteristic of those of an undulating membrane and the organism began to rotate rather rapidly. Much of the rhythmic movement observed appeared to consist of movement of the interior of the organism, rather than of the surface or of special appendages. Internal granules and nucleus in such instances oscillate with the pulsations without notable change of position of the organism as a whole and without movement of surrounding material. In one case an organism showing strong, fairly regular rhythmic movement within, continued to send out pseudopodia in various directions.

All attempts to cultivate the organism have thus far failed. The changes taking place after inoculation into the most favorable medium at present available, have been carefully followed. For thirty hours motility continues unimpaired, and the great number of motile forms gives the impression that multiplication is taking place. Binucleate forms are noted and an occasional pair of organisms suggesting binary fission, but there is subsequently no definite increase in number. Actively motile forms occur, but in progressively smaller number up to fifty-four hours in the culture tube at 37 C., but after seventy-two hours all movement has ceased. During this time the amoeboid forms, a large proportion of which show cytoplasmic granules and occasionally rather refractive globules or vacuoles, are replaced by small sized, nonmotile, spherical forms, which possess a clear hyaline cytoplasm without granules or other inclusions and a fairly readily distinguishable nucleus. It is difficult to determine whether the motile forms give rise to the hyaline resting forms or whether they die off, leaving only the latter present. The former possibility appears plausible at least as there are all transitions between the two types. The small nonmotile hyaline forms show at this time no evidence of degeneration or disintegration. No form of the parasite is to be found in culture tubes kept for five days at 37 C., although fairly well preserved organisms are present at this time in tubes kept at room temperature. These, however, show no motility when placed in the warm chamber at 41 or 42 C., and are evidently dead. Notwithstanding the great number of organisms showing amoeboid movement in the case followed in culture medium, the flagellate type of motility was not observed under the conditions furnished. With the appearance of bacteria in the culture, both motile and resting forms of the parasite quickly disappear even though previously present in great numbers. There is no trace of the parasite after twenty-four hours' incubation in salt solution to which a loopful of the cecal contents is added. The prompt disappearance of the organism in liver lesions undergoing putrefaction is also notable.

These observations are in agreement with previous morphological findings and show quite conclusively that the parasite of Blackhead is a flagellate. The rhythmic agitation of internal structures observed in certain instances may be explained only by the presence of a kinetic apparatus wholly enclosed in the cytoplasm. This in all probability consists of the previously described extranuclear body and radiating filaments which may now be interpreted as centroblepharoplast and intraprotoplasmic flagella. It is a notable fact that one of these filaments is coarser than the others (see Figs. 3 to 7, Tyzzer, 1919), but whether this represents parabasal body or trailing flagellum remains to be determined. Nothing is known concerning the possible existence of free living flagellated forms of this species, although their occurrence is suggested by the development of pulsating movements in organisms observed in the warm chamber. The recognition of such forms might possibly help to solve the problem of its transmission from host to host. It may at present only be conjectured whether such forms exist or whether the organism has become so adapted to parasitic life that it has for the most part lost its ability to live in flagellate form. Wherever, in histological preparations, it has been encountered on mucous surfaces, there is no apparent acquisition of the more characteristic flagellate type of structure. Although pulsating movement characteristic of trichomonads has been observed in material obtained from noncontaminated secondary lesions of several cases, it was not observed in similar material from another case in which amoeboid movement was maintained for fifty-four hours. The exacting conditions necessary to support this organism for even a brief period outside the body of its host as well as its prompt destruction by bacteria indicate that it possesses no marked resistance to conditions encountered outside the body of its host.

Classification.—Smith originally placed the parasite of Blackhead, on account of its amoeba-like characteristics, tentatively in the genus *Amoeba,* and much later (Smith, 1915) retained the same generic name under a different spelling *Ameba.* The view expressed by Hadley that this organism is identical with a previously described coccidium, *Eimeria avium,* is untenable, and was later abandoned by this author. Doflein's suggestion that the organism as a parasitic amoeba should be included in the genus *Entamoeba* now fails to apply with the discovery of flagellate characteristics. Both Jowett's (1911) and Hadley's (1916, 1917) incorporation of the parasite into the genus *Trichomonas* appears to be based upon a confusion of at least two intermingled species for a single species and is unacceptable without more conclusive evidence.

The proof that this organism is not an amoeba makes necessary its reclassification. Its trichomonad affinities are indicated by the type of nuclear division which it presents, by the number of flagella indicated in the five lines radiating from the blepharoplast and by the character of its pulsating movements which appear under certain conditions so that it may thus be included in the family *Tetramitidae* Saville Kent, 1880, as modified by Chalmers and Pekkola, 1918. The assumption of amoeba-like characters with respect to both movement and ingestion of solid particles together with its ability to invade vertebrate tissues appear to justify the creation of a new genus for this species. In case it should prove to be an aberrant form of a type species already described, the generic name here offered may then be suppressed. The name *Histomonas* is proposed for this genus, which may be defined as follows:

HISTOMONAS gen. nov. Pleomorphic parasitic Tetramitidae with amoeba-like phases of development within tissues of host. The kinetic structures, associated with blepharoplast, intraprotoplasmic during amoeba-like phase. Nuclear division trichomonad in type with well developed paradesmose.

Apart from the pulsating forms in hanging drop preparations of material from lesions, flagellated stages are unknown. No contractile vacuole, no cytostome observed.

Type species: *Histomonas meleagridis* (Smith, 1895) Tyzzer, 1919.

Syn. — *Amoeba meleagridis* Smith 1895.
Eimeria avium Hadley 1909.
Entamoeba meleagridis Doflein 1911.
Trichomonas eberthi Jowett 1911.
Ameba meleagridis Smith 1915.
Trichomonas Hadley 1916.

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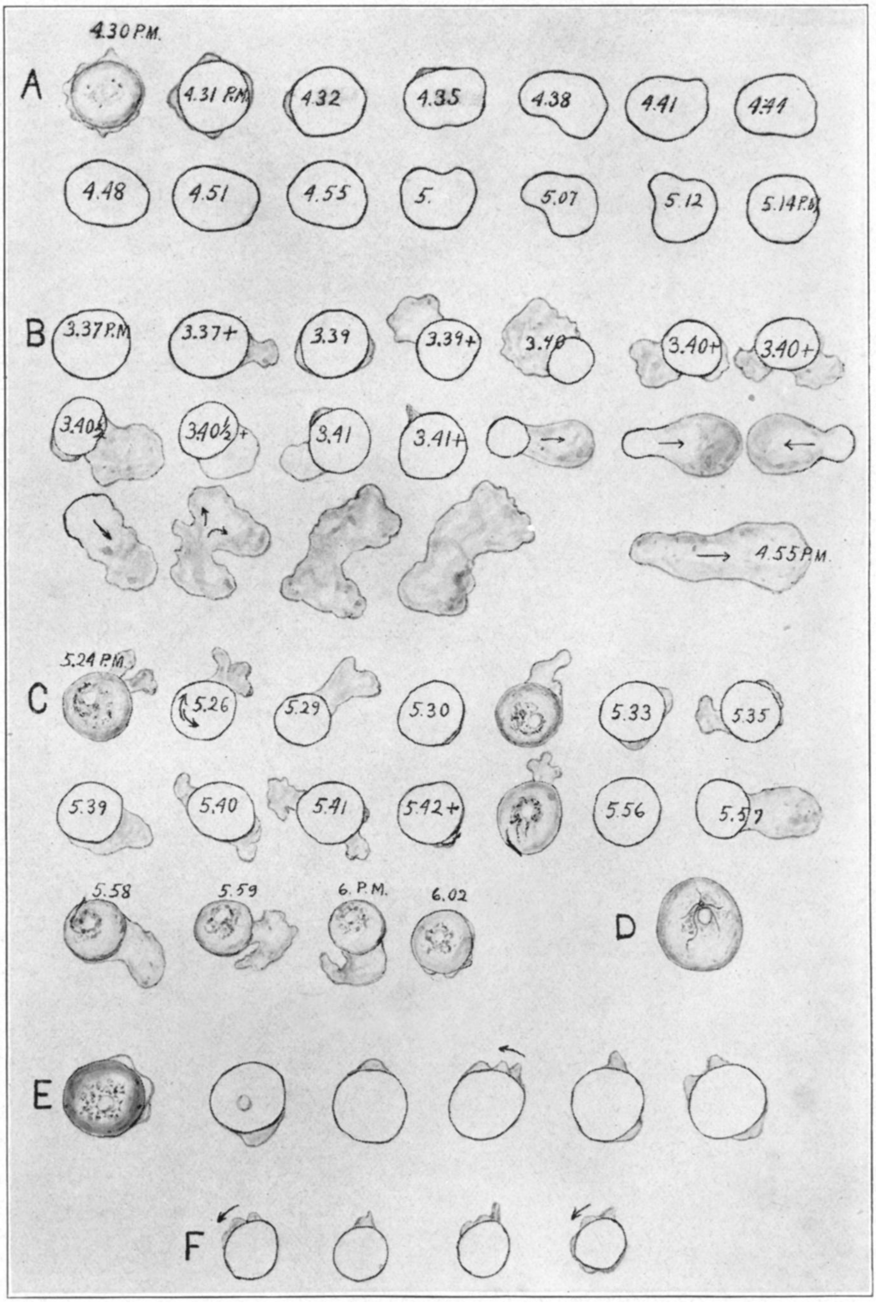
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3.40½
3.40½+
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PLATE XII

Explanation of Plate XII

Outline sketches to illustrate changes of shape in several different organisms, and in certain instances prominent structural features, observed in hanging drop preparations kept at from 41° to 42° C. in the warm chamber.

A. Different forms assumed by an organism in observations made on Nov. 7, 1919. This organism when first seen showed wave-like pseudopodia. After retraction of the pseudopodia the organism then showed merely slow change of form.

B. Changes of form in an organism observed on Nov. 15, 1919. There was great rapidity of movement with pseudopodia quickly extended and retracted at times from various portions of the surface. The organism eventually spread out in a very thin sheet against the cover glass and moved about rapidly, showing an irregular outline except for a certain period, when it assumed a slug-like form. No pulsation was observed. The nearest approach to this was a quick reversal of movement as indicated in the three sketches following that illustrating the form assumed at 3:41 p. m. This reversal was repeated several times.

C. An organism studied on Nov. 15, 1919. Regular pulsation was noted on first finding the organism about three hours after the preparation was made. The movement consisted of a rotary pulsation of the interior of the organism occurring at regular intervals. Pseudopodia continued to be actively protruded throughout the observation. As the organism rotated upon itself, activity was noted at one point in its surface where there was a slender projecting structure, probably a rudimentary flagellum. This was plainly seen whipping in the surface of the organism at 5:56 p. m., but is not shown in sketch.

D. An organism observed in fresh preparation on Dec. 3, 1919. An extranuclear granule with five radiating lines is visible. In this material no pulsating forms were observed, although the organisms showed active amoeboid motion for fifty-four hours.

E. A parasite followed in observations made on Nov. 7, 1919. First wave-like pseudopodia were observed and in a short time the movement of these became rhythmical and the organism commenced to rotate rapidly.

F. A small organism observed on preceding date of observation. This was first seen sending out rather sharp, wave-like pseudopodia. These traveled in one direction and the organism soon began to pulsate regularly and to rotate actively.